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CHROMOSOME COUNTS FOR *PACKERA PAUPERCULA* VAR.
APPALACHIANA (ASTERACEAE: SENECEONEAE)

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ABSTRACT

Certain populations of more or less tomentose *Packera* (Asteraceae: Senecioneae) in southeastern North America have recently been treated as *P. paupercula* var. *appalachiana* based on evidence from a common garden, canonical discriminant analyses of morphological characters and flowering date, a preliminary RAPDs analysis, and crosses. Previously they had been treated as disjuncts of *P. plattensis*, a species of the Great Plains, which may have migrated into the mid Appalachians during the post-glacial Hypsithermic Period and survived there as relicts. However, *P. plattensis* is tetraploid with $n = 46$ and $x = 23$. Our two chromosome counts of the new variety from North Carolina and from Virginia are $n = 22$ and $n = ca. 44$, respectively, a diploid and a tetraploid, both with $x = 22$. Statistical analyses of pollen diameter provide evidence that the other individuals in the two populations are diploid and mostly tetraploid, respectively. Hybridization with *P. aurea* may be involved in the origin of the tetraploid. Thus, cytological evidence supports other data showing that these populations may be more closely related to *P. paupercula*.

RESUMEN

Ciertas poblaciones de *Packera* (Asteraceae: Senecioneae) más o menos tomentosas del sureste de Norte América han sido tratadas recientemente como *P. paupercula* var. *appalachiana* basándose en evidencias procedentes de un jardín común, análisis canónico discriminante de caracteres morfológicos y datos florales, un análisis preliminar de RAPDs, y cruces. Previamente habían sido tratados como disyuntas de *P. plattensis*, una especie de las Grandes Llanuras, que podría haber migrado a los Apalaches medios durante el periodo hipsitérico post-glacial y sobrevivido allí como relicto. Sin embargo, *P. plattensis* es tetraploide con $n = 46$ y $x = 23$. Nuestros dos recuentos cromosómicos de la nueva variedad de Carolina del Norte y de Virginia son $n = 22$ y $n = ca. 44$, respectivamente, un diploide y un tetraploide, ambos con $x = 22$. Los análisis estadísticos del diámetro del polen evidencian que los otros individuos de las dos poblaciones son diploides, y preferentemente tetraploides, respectivamente. La hibridación con *P. aurea* puede ser la causa del origen del tetraploide. Así, la evidencia citológica apoya otros datos que muestran que estas poblaciones pueden estar más emparentadas con *P. paupercula*.

INTRODUCTION

Packera plattensis (Nutt.) Weber and Á Löve is distributed in dry grasslands from the eastern flanks of the southern Rocky Mountains to the Mississippi

River with scattered populations eastward in Wisconsin and Missouri (Barkley 1962, 1978) (Fig. 1). It is tetraploid with a base chromosome number of $x = 23$ (Kowal 1975; Kowal et al. unpubl. ms.).

Apparently disjunct populations occur in Pennsylvania, Virginia, Tennessee, North Carolina and South Carolina (Barkley 1962, 1978, 1988; Uttal 1982; Rhoads & Klein 1993) (Fig. 1). Barkley hypothesized that these are remnants of the widespread eastern migration of prairie-adapted plants during the Hypsithermic Period of about 8000 years ago. Such populations in Montgomery Co., Virginia commonly hybridize with *P. aurea*, *P. anonyma*, and *P. obovata* (Uttal 1982). Because the latter three species are usually diploid with $x = 22$ (Kowal et al. unpubl. ms.), widespread hybridization with *P. plattensis* would be unexpected (Mahoney & Kowal ms. subm.; Kowal et al. unpubl. ms.). Moreover, herbarium specimens from the entire range of the eastern disjunct populations are morphologically different from *P. plattensis* of the Great Plains in their rosette and cauline leaves and in their smaller heads.

Individuals from North Carolinian and Virginian populations of putative *P. plattensis* were included in a larger study of the *P. paupercula* complex and allied species (Mahoney & Kowal ms. subm.). Replicates were grown in a standard environment transplant garden, and data on morphology and flowering time were analyzed by canonical discriminant analyses (CDA) and analyses of variance (ANOVA). Herbarium specimens of the complex, emphasizing its distribution east of the Mississippi River, were studied, and a preliminary analysis of genetic similarity using random amplified polymorphic DNAs (RAPDs) was made. One conclusion is that these central Appalachian populations are best included in the polymorphic *P. paupercula* complex as *P. paupercula* var. *appalachiana* A.M. Mahoney var. nov. ined. Chromosome numbers provide critical evidence supporting this conclusion.

MATERIALS AND METHODS

Individuals of putative eastern disjunct *Packera plattensis* (*P. paupercula* var. *appalachiana*) were collected from two sites. The Paint Rock Road population (NC, Madison Co.: near Hot Springs, 3.2 mi northwest along Paint Rock Road from Hwy 25-70, Kowal & Mahoney 3120 [WIS]) grows in loose, gravelly soil on a steep, wooded, southwest-facing slope and road cut overlooking the French Broad River. The Lover's Leap population (VA, Montgomery Co.: 2 mi west from Price's Fork on McCoy Road [rt. 652], Kowal & Mahoney 3123 [WIS]) consists of scattered clones on exposed and wooded rocky outcrops on a south-facing bluff overlooking the New River and along the south side of McCoy road on a gravelly bank. Replicated individuals were randomly positioned in a standard-environment transplant garden in Madison, WI.

Heads from test garden individuals were fixed in Jackson's solution (4 ethanol: 2 methanol: 2 chloroform: 1 propionic acid: 1 acetone, v/v), transferred

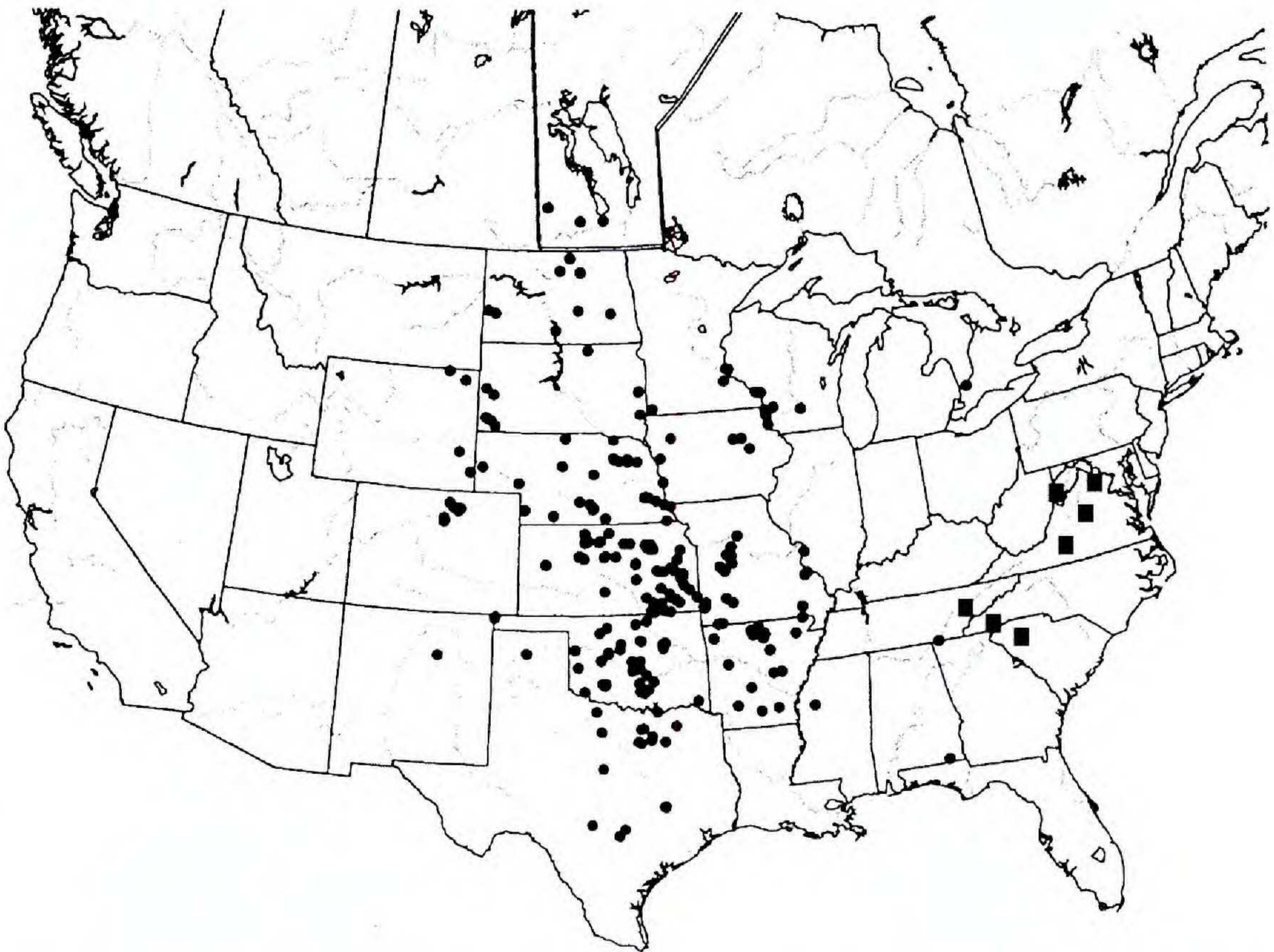


FIG. 1. Distribution of *Packera plattensis* and hypothesized disjunct populations in Virginia, North Carolina, and South Carolina (*P. paupercula* var. *appalachiana*). ● *P. plattensis*; ■ Disjunct populations (*P. paupercula* var. *appalachiana*).

after about a day to 70% ethanol, and stored in a freezer. Counts were made on meiocytes of young anthers from florets 2.5–3.0 mm in length. Using a squash technique, described in Beeks (1955) as modified by Kowal (1975) and Kong (1998), anthers were prestained in aceto-carmin and squashed between a slide and a cover slip. Each slide was scanned under the 40 × objective of a Zeiss Standard WL phase-contrast microscope, and cells with promising meiotic figures were resquashed until their chromosomes were well-separated and in one plane. Coverslips were sealed with “Zut.” Counts were made with the 100 × objective under oil immersion, and cells with well-separated chromosomes were photographed with Kodak Ektachrome 160 and Technical Pan (exposed at an ASA of 100) films using a Nikon Microflex Model AFM photomicrographic attachment (Kong 1998). Results are in Table 1 and substantiating photographs are in Figures 2 and 3.

RESULTS

Individual 9 from Paint Rock Road (NC, Madison Co.) is diploid with $n = 22$ (Kong 1998; Figs. 2 & 3). Individual 9 from Lover’s Leap (VA, Montgomery Co.)

TABLE 1. Chromosome counts for *Packera paupercula* var. *appalachiana*.

Taxon	Haploid number	Cells counted	Phase
NC, Paint Rock Rd, #9	22	34	Metaphase I & II
VA, Lover's Leap, #9	44	1	Anaphase I

is tetraploid with $n = 44$, based on a single, but fairly good preparation (Figs. 4 & 5). A poor anaphase I from individual 10 from Lover's Leap gave an extremely rough count of $2n = \text{ca. } 67$, superficially, an approximately triploid count. However, the regular meioses, terminating in normal tetrads of nuclei without micronuclei, in this individual prompts the interpretation that individual 10 is tetraploid as well.

Pollen diameters from the transplant garden can be used to extend the counts of the two individuals to their respective populations, because pollen diameter is correlated with polyploid level (Kowal 1975; Mahoney & Kowal ms. subm.). A nested ANOVA indicates that the difference in mean pollen diameter between the North Carolinian and Virginian populations, 27.9 vs. 31.2 μm , is real ($F_{1,8} = 24.16$, $p = 0.0018$) and that individuals within each population do not differ ($F_{8,7} = 2.19$, $p = 0.16$). Taken together, this implies that the populations, the former containing an individual with $n = 22$ and the latter with $n = \text{ca. } 44$, consist of all diploids and all tetraploids, respectively. ANOVAs testing for differences among individuals within the two populations separately give absolutely no evidence ($F_{5,7} = 0.46$, $p = 0.80$) of differences in pollen diameter (and, implicitly chromosome number) in the "diploid" population but moderate evidence ($F_{3,7} = 5.11$, $p = 0.035$) in the "tetraploid" population. However, the one individual with unusually small pollen diameter (29.0 μm) is, in fact, individual 10, which is approximately tetraploid, so its aberrancy has other causes.

Uttal (1982) notes that among the specimens from Madison Co., NC (presumably the Paint Rock Road population), he saw hybrids with *P. anonyma*. This species does occur ca. 0.5 miles downstream from the var. *appalachiana* population, and 3 of the 11 individuals collected in 1995 showed morphological similarities to *P. anonyma*. Introgression here is likely, but none of the individuals in the transplant garden provided any obvious evidence of it.

Uttal (1982) reports that *P. aurea* and *P. paupercula* var. *appalachiana* (as "*P. plattensis*") "thoroughly introgress" along McCoy Road (rt. 652). Individual 10 grew on the road cut and individual 9 grew nearby, on the bluff's edge; the other individuals were from less disturbed bluff sites. Some individuals, in particular #9, have deeply pinnatifid cauline leaves with wide, distally dilated lobes and broad, undivided terminal lobes like *P. aurea*, which is rampant in an apparent sink-hole in the woodland above the bluff. A garden-grown individual of *P. aurea* from this site is lighter green than is typical for the species and



FIG. 2. NC, Paint Rock Road, #9. Metaphase I, $2n = 22$ II.



FIG. 3. NC, Paint Rock Road, #9. Metaphase II, $2n = 22 + 22$.

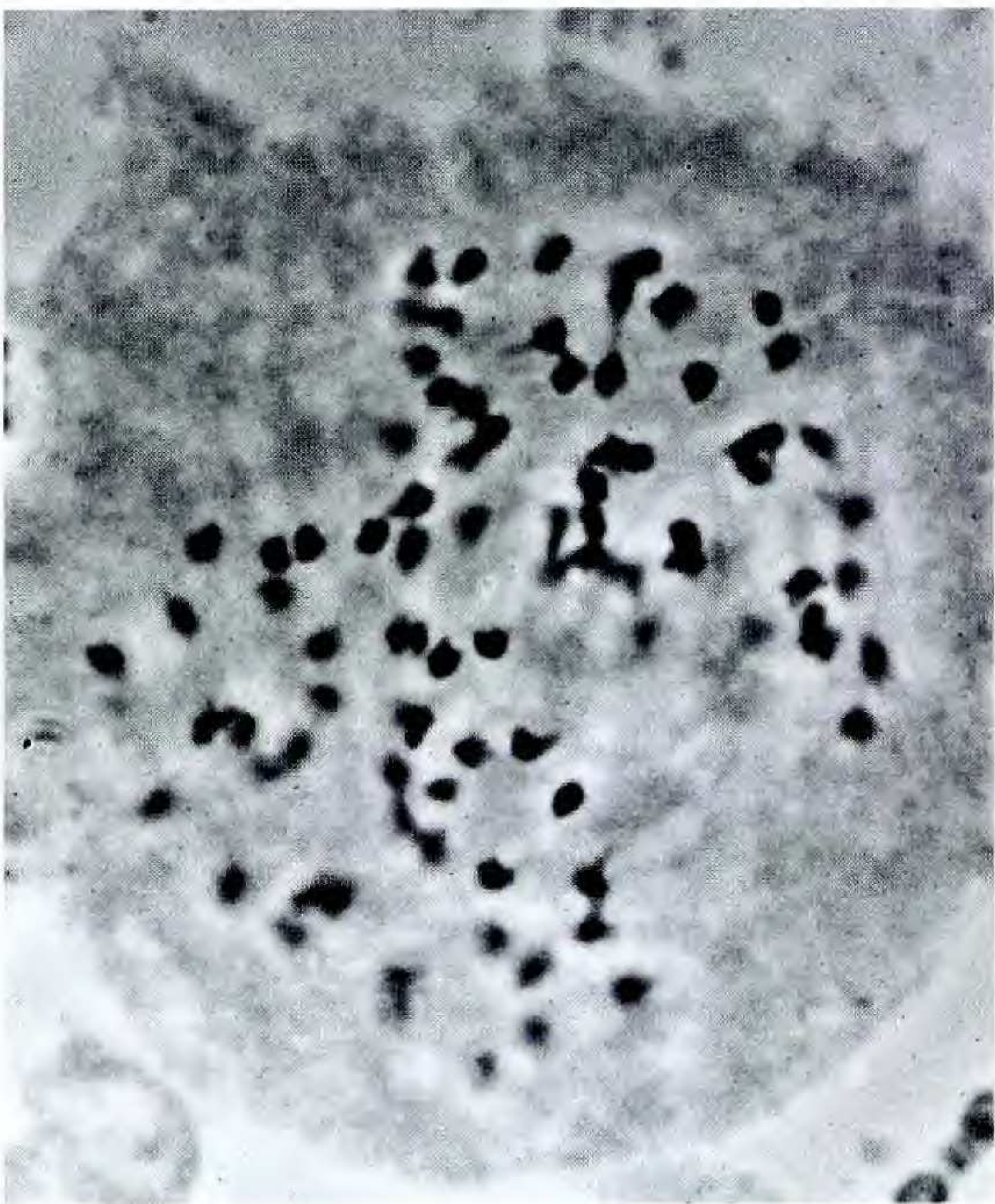


FIG. 4. VA, Lover's Leap, #9. Late anaphase I, $2n = 22 + ca, 88$.

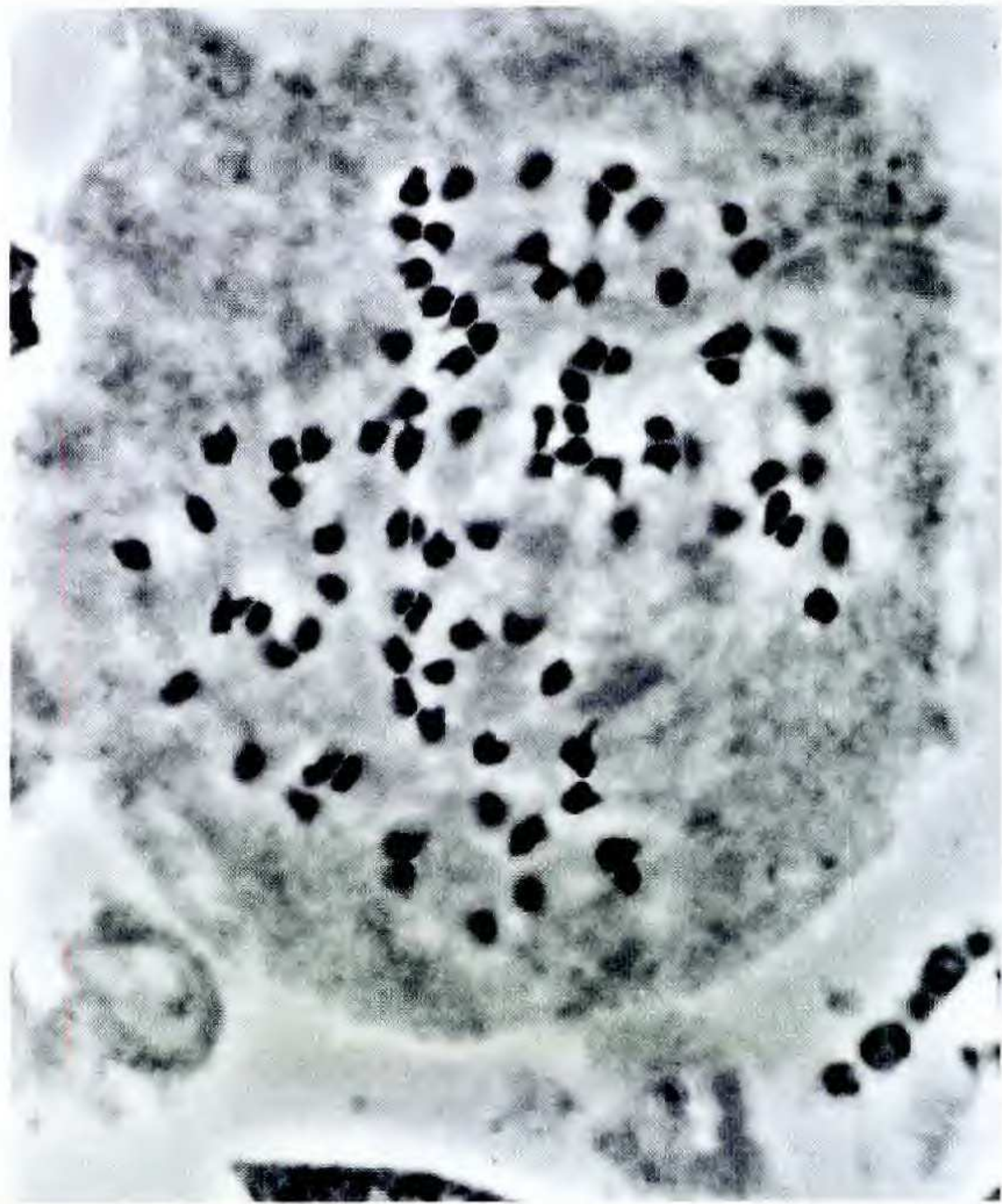


FIG. 5. Figure 3 retouched using Corel Photo-Paint 8.

reproduces abundantly and invasively by adventitious shoots on roots, unlike normal *P. aurea*. Therefore, it is likely that at least a portion of the Lover's Leap population is introgressed with *P. aurea*.

Chromosome counts indicate that *Packera plattensis* does not occur in the central Appalachians. This Great Plains species appears to be entirely tetraploid with $x = 23$ and the North Carolinian population is diploid with $x = 22$. At least some members of the Lover's Leap population in Virginia are tetraploid but probably with $x = 22$. This evidence supports the morphological and RAPDs evidence that these central Appalachian populations are best treated as *P. paupercula* var. *appalachiana* (Mahoney & Kowal ms. subm.).

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